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Effect of treatment with vitamin D_3 on the responses of the duodenum of spontaneously hypertensive rats to bradykinin and to potassium

Teresa Feres, Lucia M. Vianna, Antonio C.M. Paiva & [1]Therezinha, B. Paiva

Department of Biophysics, Escola Paulista de Medicina, 04034, São Paulo, SP, Brazil

1 The diet of spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto (WKY) and Wistar (NWR) rats was supplemented with either 2% calcium lactate in the drinking water or 12.5 μg vitamin D_3 100 g^{-1} body weight daily by gavage, for 14 days.

2 The blood pressure of the SHR treated with either calcium or vitamin D decreased to the same levels as that of WKY and NWR.

3 The response to bradykinin of the SHR isolated duodenum, which is predominantly contractile, upon treatment with vitamin D (but not with calcium), became predominantly relaxant, approaching the normal behaviour of the WKY and NWR duodenum.

4 The relaxant responses of the SHR and WKY duodenum to potassium were smaller than those of NWR, but treatment with vitamin D increased the response in all three rat strains.

5 It is concluded that, besides sharing the hypotensive effect of calcium, vitamin D treatment of SHR has an effect on the duodenum smooth muscle which might be due to calmodulin-dependent activation of calcium-dependent potassium channels.

Keywords: Bradykinin; duodenum; intestinal smooth muscle; spontaneously hypertensive rats; vitamin D

Introduction

Several studies have shown that an altered calcium metabolism may play a significant role in the development and maintenance of hypertension both in man and in spontaneously hypertensive animals (SHR) (for a review, see McCarron, 1989). In the SHR, vitamin D metabolism was reported to be abnormal (Lucas *et al.*, 1986) and calcium supplementation in the diet caused blood pressure reduction (Ayashi, 1979; Bukoski & McCarron, 1986). More recently, dietary supplementation with vitamin D_3 was shown to reduce arterial blood pressure in the SHR (Vianna *et al.*, 1992). Apparently, a disturbance in vitamin D_3 and calcium metabolism is linked to the hypertensive mechanism in SHR (Lucas *et al.*, 1986).

Calcium is also known to be associated with the electrophysiological stability of the cell membrane, being important for the relaxation mechanism in smooth muscle (Rinaldi & Bohr, 1989). However, in spite of numerous studies on the vascular reactivity in SHR, controversial results have been obtained, depending on the kind of vascular smooth muscle used (for a review see Bohr & Webb, 1988).

An interesting model for studying smooth muscle relaxation in the rat is the response of the duodenum to bradykinin. In normal rats, the response to low concentrations of this agonist consists only of a relaxation, whereas higher concentrations elicit relaxation followed by contraction (Boschcov *et al.*, 1984). Previous work from this laboratory showed that the response of the SHR to bradykinin is predominantly contractile, with suppression of its relaxant component (Miasiro *et al.*, 1985). We have, therefore, investigated whether dietary supplementation with vitamin D_3, under conditions that cause reversion of the hypertension in SHR, is also able to reverse the abnormal behaviour of the duodenum of these animals towards bradykinin. In this paper, we present the results of a study on the effect of treatment with vitamin D_3 on the responses of SHR and of normotensive Wistar Kyoto rats (WKY), as well as normotensive Wistar rats (NWR).

Methods

Experiments were carried out on the Okamoto-Aoki strain of SHR, their normotensive controls (WKY), and normotensive Wistar rats (NWR). The animals were female aged 20 weeks and weighed 200 ± 5 (SHR), 220 ± 7 (WKY), 198 ± 3 g (NWR). They were fed a standard diet (Labina rat chow, Purina), containing 6,600 i.u. vitamin D_3 kg^{-1}. After a basal period of 10 days, one group of animals were submitted to a daily supplementation, by gavage, of 12.5 μg (500 i.u.) vitamin D_3 per 100 g body weight, dissolved in 0.35 ml coconut oil. In another group the drinking water was replaced by a 2.0% calcium lactate aqueous solution. Control groups had access to normal drinking water, and one of them received 0.35 ml coconut oil by gavage, daily.

Systolic blood pressure was measured twice weekly from the tail of prewarmed unanesthetized rats by a plethysmographic method. An average of three readings was recorded for each animal.

The rat duodenum preparation was set up as previously described (Boschcov *et al.*, 1984). Briefly, the duodenum was suspended in a 5 ml chamber containing a salt solution of the following composition (mM): NaCl 137, KCl 2.7, $CaCl_2$ 1.36, $MgCl_2$ 0.49, NaH_2PO_4 0.36, $NaHCO_3$ 11.9, D-glucose 5.0. The bath solution was maintained at 37°C and bubbled with a mixture of CO_2 (5%) and O_2 (95%). The organs were submitted to a 1 g load and their isotonic contractions were recorded (with a 6 fold magnification) after a 60 min equilibration period.

The concentration-response curves were obtained within the first 90 min after the end of the equilibration period. The drugs, in volumes not exceeding 0.2 ml, were added directly

[1] Author for correspondence.

to the organ bath at 5 min intervals for KCl, at 15 min intervals for low bradykinin concentrations (which produced relaxation) and at 30 min intervals for higher bradykinin concentrations (which caused also contractile responses, with a slower recovery of the normal responsive state). The preparation was washed after a 90 s contact time with either agent.

The relaxant component of the response was measured from the baseline to the deepest point of the relaxation and the contractile component from the baseline to the highest point of the response. The dose-response curves were analyzed by linear regression of the double-reciprocal plot, from which ED_{50} values were obtained.

Bradykinin was a synthetic product made in this laboratory (Sabia *et al.*, 1977). The inorganic salts were products of the highest analytical grade from Merck Darmstadt. Vitamin D_3 (cholecalciferol) was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.

All data were expressed as means ± s.e.means and were analyzed by Student's *t* test.

Results

In the SHR, low bradykinin concentrations elicited only small relaxations that did not show concentration-dependence in the range 0.4–2 nM (Figure 1a). At bradykinin concentrations of 4 nM and above, the SHR duodenum responded with a concentration-dependent contraction preceded by a small relaxation. This predominantly contractile response of the SHR duodenum contrasted with the predominantly relaxant response of the NWR and WKY duodenum, in agreement with a previous report (Miasiro *et al.*, 1985). The typical responses shown in Figures 1b (WKY) and 1c (NWR) show a concentration-dependent relaxation in the range 0.4 nM, above which the responses consisted of a maximum relaxation followed by a concentration-dependent contraction. Concentration-response curves obtained in these experiments yielded the ED_{50} values listed in Table 1.

The SHR which received 2% calcium lactate in the drinking water, or a daily diet supplementation with 12.5 μg vitamin D_3, had their blood pressure reduced to normal levels, whereas no significant changes were observed in the blood pressure of WKY animals submitted to the same treatment (Figure 2).

In the calcium-treated SHR, the responses of the duodenum to bradykinin remained predominantly contractile, with a minor relaxant component which was not dose-dependent, similar to that seen in non-treated SHR (compare Figures 1a and 3b). However, a marked change was observed in the behaviour of the duodenum preparations of vitamin D_3-treated SHR (Figure 3a), which became similar to those of the NWR and WKY preparations, with concentration-dependent relaxation in the range 0.4–40 nM, and appearance of the contractile component of the response only at concentrations above 4 nM. These changes were evident by a comparison of the concentration-response curves in the treated and control groups, summarized by the ED_{50} values shown in Table 1.

It is interesting to note that the vitamin D_3 treatment also changed the responses of the duodenum from the normotensive controls. The ED_{50} values for the relaxant component of the responses of both NWR and WKY rats were significantly decreased after the treatment (Table 1). As for the contractile component, the duodenum of the vitamin D_3-treated WKY, but not that of the NWR, presented a shift to the right on

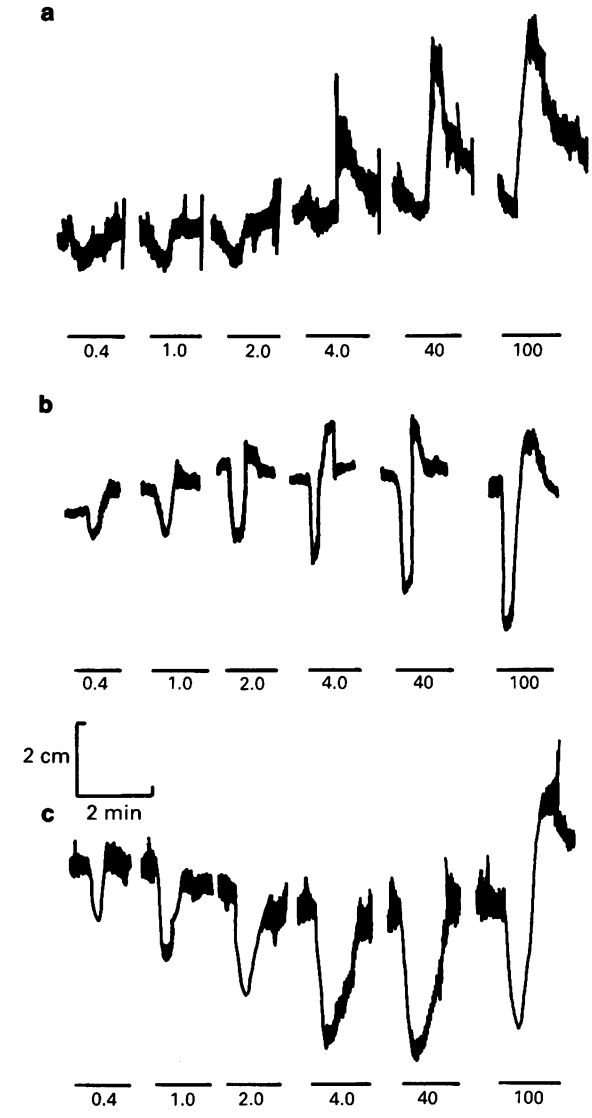

Figure 1 Responses of three rat duodenum preparations from SHR (a), WKY (b), and NWR (c) to different concentrations (in nM) of bradykinin. Each treatment lasted for 90 s (horizontal bar) and was followed by washing and a resting period of 13.5 min (indicated by the interruptions in the tracings). The amplitude of the isotonic responses was magnified 6 fold. These results are representative of those obtained in at least 8 experiments.

Table 1 ED_{50} values for the relaxant and contractile components of the responses of rat duodenum before and after treatment with vitamin D_3

	Relaxation		*Contraction*	
	Before treatment	*After treatment*	*Before treatment*	*After treatment*
NWR	$6.76 \times 10^{-9} \pm 0.6$	$5.88 \times 10^{-10} \pm 0.16$*	$1.58 \times 10^{-7} \pm 0.9$	$1.00 \times 10^{-7} \pm 0.87$
SHR	ND	$4.89 \times 10^{-9} \pm 0.79$	$3.02 \times 10^{-9} \pm 0.81$	$5.01 \times 10^{-8} \pm 0.81$*
WKY	$1.62 \times 10^{-8} \pm 0.79$	$5.89 \times 10^{-10} \pm 0.32$*	$5.13 \times 10^{-9} \pm 0.76$	$1.58 \times 10^{-8} \pm 0.76$*

Values are means ± s.e.means of 6–8 experiments. *Significantly different from values before treatment ($P < 0.5$); ND indicates that ED_{50} was not determined because the effect was not concentration-dependent.

the bradykinin dose-response curves after the treatment.

Duodenum preparations from SHR and WKY presented an impaired relaxant response to K^+ that was interpreted as a result of the inhibition of the Na^+/K^+ pump activity in these animal strains, as compared with the NWR (Miasiro *et al.*, 1985). After vitamin D_3 treatment, duodenum from all three strains presented a significant increase of the relaxant response to K^+ (Figure 3).

Discussion

The blood pressure of SHR was shown to be lowered by chronic dietary supplementation with either calcium (Ayachi, 1979; Bukoski *et al.*, 1986) or vitamin D_3 (Vianna *et al.*, 1992). The mechanism involved in the hypotensive effect of calcium supplementation in the SHR has been the subject of much investigation (for a review, see Bukoski *et al.*, 1986), but more recent evidence suggests that the lowering of the blood viscosity due to a decreased haematocrit may play a major role in this mechanism (Susic *et al.*, 1984).

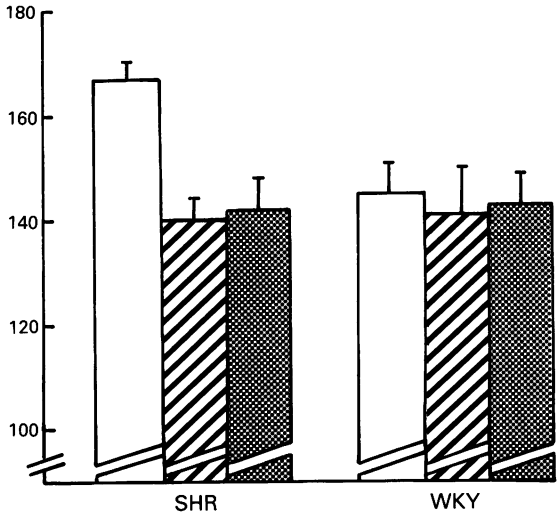

Figure 2 Effect of treatment with vitamin D_3 or calcium on the blood pressure of SHR and WKY rats. Open columns, controls; hatched columns after supplementation with 12.5 μg vitamin D_3 100 g^{-1} body weight daily; stippled columns, after 14 days receiving 2% calcium lactate in the drinking water. Data are means of 6 animals and the s.e.mean is indicated by vertical bars.

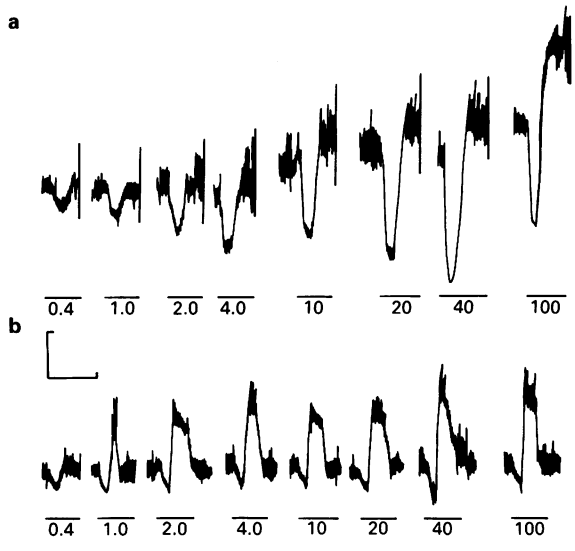

Figure 3 Responses of two rat duodenum preparations from SHR after treatment for two weeks with: (a) 12.5 μg vitamin D_3 100 g^{-1} body weight daily, by gavage; (b) 2% calcium lactate in the drinking water. The indicated concentrations of bradykinin (in nM) were applied for 90 s (horizontal bars), followed by washing and a resting period of 13.5 min (interruptions in the tracings). The amplitude of the isotonic responses was magnified 6 fold.

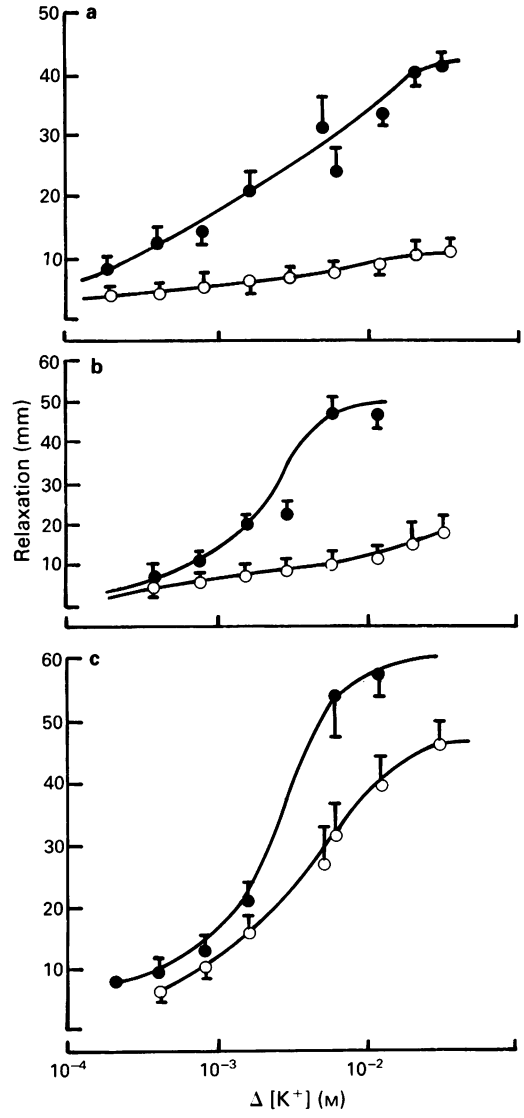

Figure 4 Concentration-response curves for the relaxant component of the duodenum response to KCl in duodenum preparations isolated from SHR (a), WKY (b) and NWR (c): (●) treated with 12.5 μg vitamin D_3; (○) controls.

In the present work we have investigated how dietary supplementation with calcium or vitamin D affects the abnormal behaviour of the SHR duodenum towards the effects of bradykinin. We have found that, although both treatments are equally effective in lowering the blood pressure in these animals, only the supplementation with vitamin D_3 changed the duodenum response from predominantly contractile to predominantly relaxant, making the behaviour of preparations from vitamin D_3-treated SHR indistinguishable from those of the normotensive controls. It may be concluded that the vitamin treatment besides having a mechanism in common with that of calcium supplementation with respect to blood pressure lowering, affects the duodenum responses through a different mechanism.

In duodenum preparations of normotensive rats, bradykinin has a predominantly relaxant effect, a contractile

component becoming evident only at high concentrations, in which a biphasic response is observed, namely, a maximum relaxation followed by contraction. Previous work has indicated that the relaxant and contractile components of the response are respectively due to activation of B_2 and B_1 receptor populations (Paiva *et al.*, 1989).

The mechanism underlying the relaxant effect induced by bradykinin in several smooth muscle preparations has been shown to involve hyperpolarization due to the opening of apamin-sensitive calcium-activated K^+ channels (Carter *et al.*, 1986; Hall *et al.*, 1991). The activation of these channels depends on calmodulin levels (Pershadsingh *et al.*, 1986; Okada *et al.*, 1987), which are depressed in several tissues of the SHR (Baba *et al.*, 1987). Since vitamin D promotes the synthesis of several calcium-binding proteins, including calmodulin (Gross & Kumar, 1990; Fernandez *et al.*, 1990), it is conceivable that its effect on the relaxant component of the bradykinin response may be due to an increase in the calmodulin content in the SHR duodenum and consequent increase in the responsivity of calcium-activated K^+ channels.

Concerning the contractile component of the response, Boschcov *et al.* (1984) have proposed that a deficient calcium handling by the cell membrane in SHR favours both an increased Na^+ conductance and the duodenum contraction induced by high bradykinin concentrations. Vitamin D_3 treatment, by increasing the binding of calcium to the cell membrane promotes membrane stability (Bukoski *et al.*, 1986) decreasing Na^+ conductance and consequently might be responsible for the decrease in the contractile component of the responses to bradykinin in the vitamin D-treated SHR. In addition, the decrease of the contractile component of the response of the duodenum of vitamin D-treated SHR may in part be due to the increase in the relaxant component, since the biphasic response of gastrointestinal smooth muscle preparations appears to reflect a balance between two opposing actions (Carter *et al.*, 1985).

The relaxation induced by KCl, which is impaired in SHR, was also normalized after the vitamin D_3 treatment. However, this was a generic effect, since the duodenum of NWR and WKY also showed a significant increase in the relaxant component of the response to potassium. The relaxation induced by the increase of extracellular potassium is attributed to hyperpolarization due to the stimulation of the Na^+/K^+ pump (Rinaldi *et al.*, 1989). The decreased relaxant response of the SHR and WKY duodenum to potassium confirmed previous findings from our laboratory which showed an inhibition of the Na^+/K^+ pump in this strain (Miasiro *et al.*, 1985). Our results therefore suggest that the normalization of the relaxant response of the SHR by chronic treatment with vitamin D_3 may be due to an activation of this pump by vitamin D. The normalization of the Na^+/K^+ pump activity could also contribute to the hypotensive effect of vitamin D_3, since Bukosi *et al.* (1986) has already suggested a link between Na^+/K^+ pump activity normalization and the hypotensive effect of dietary calcium.

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